

## PARENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION  
(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
 United States Patent and Trademark  
 Office  
 Box PCT  
 Washington, D.C.20231  
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 04 August 2000 (04.08.00)	Assistant Commissioner for Patents United States Patent and Trademark Office Box PCT Washington, D.C.20231 ETATS-UNIS D'AMERIQUE
International application No. PCT/US99/24018	Applicant's or agent's file reference F134322
International filing date (day/month/year) 12 November 1999 (12.11.99)	Priority date (day/month/year) 13 November 1998 (13.11.98)
Applicant MCGUINNESS, Ryan et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

31 May 2000 (31.05.00)

in a notice effecting later election filed with the International Bureau on:

\_\_\_\_\_

2. The election  was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer F. Baechler
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

## PATENT COOPERATION TREATY

PCT

NOTICE INFORMING THE APPLICANT OF THE  
COMMUNICATION OF THE INTERNATIONAL  
APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year)
25 May 2000 (25.05.00)

Applicant's or agent's file reference
F134322

From the INTERNATIONAL BUREAU

To:  
 NAKAMURA, Dean, H.  
 Sughrue, Mion, Zinn, MacPeak &  
 Seas, PLLC  
 Suite 800  
 2100 Pennsylvania Avenue  
 Washington, DC 20007-3213  
 ETATS-UNIS D'AMERIQUE

Doc'd \_\_\_\_\_ File \_\_\_\_\_  
 JUN 2 2000  
 ROYLANCE, ABRAMS  
 BERDO & GOODMAN, L.L.P.  
 BY \_\_\_\_\_

## IMPORTANT NOTICE

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/US99/24018	12 November 1999 (12.11.99)	13 November 1998 (13.11.98)
Applicant CELL GENESYS, INC. et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:

AU,CN,JP,KP,KR,MA,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,  
 GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,  
 PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on  
 25 May 2000 (25.05.00) under No. WO 00/29421

## REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

## REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO  
 34, chemin des Col mbettes  
 1211 Geneva 20, Switzerland

Facsimile N. (41-22) 740.14.35

Authorized officer

J. Zahra

Telephone No. (41-22) 338.83.38

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/24018

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07H 21/00, 21/04; C12N 15/00, 15/09, 15/63, 15/70, 15/74  
US CL : 435/320.1; 536/23.1, 23.7, 23.72

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1; 536/23.1, 23.7, 23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, DIALOG, EMBASE, MEDLINE, BIOSIS, SCISEARCH

search terms:nucleic acids, construct, promoter, donor site, gag, pol, HIV, marker, expression cassette, vector, REV

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,739,118 A (CARRANO et al) 14 April 1998, columns 33-34.	1 and 2
X	US 5,716,613 A (GUBER et al.) 10 February 1998, columns 11-12.	1 and 2
X	US 5,716,826 A (GRUBER et al.) 10 February 1998, columns 11-12.	1 and 2
Y	US 5,747,307 A (LEVER et al.) 05 May 1998, figs. 4-5, columns 7-12.	1 and 2
Y	US 5,693,508 A (CHANG) 02 December 1997, columns 45-48.	1 and 2

 Further documents are listed in the continuation of Box C. See patent family annex.

Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 FEBRUARY 2000

Date of mailing of the international search report

15 FEB 2000

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT

Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

BRETT L. NELSON

Telephone No. (703) 308-0196

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7:  
C07H 21/00, 21/04, C12N 15/00, 15/09,  
15/63, 15/70, 15/74

A1

(11) International Publication Number:

WO 00/29421

(43) International Publication Date:

25 May 2000 (25.05.00)

(21) International Application Number: PCT/US99/24018

(22) International Filing Date: 12 November 1999 (12.11.99)

(30) Priority Data:  
60/108,169 13 November 1998 (13.11.98) US

(71) Applicant (for all designated States except US): CELL  
GENESYS, INC. [US/US]; 342 Lakeside Drive, Foster  
City, CA 94404 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): MCGUINNESS, Ryan  
[US/US]; Cell Genesys, Inc., 342 Lakeside Drive, Foster  
City, CA 94404 (US). NALDINI, Luigi [IT/IT]; Cell  
Genesys, Inc., 342 Lakeside Drive, Foster City, CA 94404  
(US).

(74) Agents: NAKAMURA, Dean, H. et al.; Sughrue, Mion, Zinn,  
MacPeak & Seas, PLLC, Suite 800, 2100 Pennsylvania  
Avenue, Washington, DC 20037-3213 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG,  
BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE,  
ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP,  
KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,  
MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU,  
SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG,  
US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE,  
LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM,  
AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT,  
BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,  
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

(54) Title: SELECTION SYSTEM FOR GENERATING EFFICIENT PACKAGING CELLS FOR LENTIVIRAL VECTORS

(57) Abstract

A method for selecting packaging cells that express high levels of gag/pol is provided.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

# Selection System For Generating Efficient Packaging Cells For Lentiviral Vectors

## BACKGROUND OF THE INVENTION

Generation of efficient packaging cell lines for lentiviral vectors is hampered by the cytotoxicity of some of the products of the gag and pol genes. Thus, it is desirable to have inducible expression of gag and pol so that optimal clones that will express gag and pol at high levels when needed can be selected in the absence of gag/pol expression.

5

## SUMMARY OF THE INVENTION

A method for selecting cells which express gag and pol and thus are useful as packaging cells is obtained by linking a selectable marker to the gag/pol expression cassette of a packaging vector in such a way that the marker is expressed by the same promoter which controls expression of the gag/pol genes although expression of the gag/pol genes is suppressed. Efficient expression of the marker predicts efficient expression of the gag/pol genes on induction.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a recombinant vector which exemplifies the instant invention. The gag/pol sequences are flanked by splice donor and splice acceptor sites. Also contained within the splice donor and splice acceptor sites is an RRE (Rev responsive element).

Figure 2 depicts the mechanism by which a vector containing an RRE would provide inducible expression of only the marker gene, in the case of the exemplary recombinant vector of Figure 1, the marker is CD4. In the absence of Rev, splicing occurs between the splice donor and splice acceptor sites thereby eliminating the gag/pol sequence.

Only CD4 is expressed. When Rev is present, splicing does not occur and the gag/pol genes are expressed.

Figure 3 is a graph depicting the amounts of p24, a product of the gag gene, in culture medium when cells containing a vector of the instant invention are propagated in the presence or absence of Rev. Two different vectors were used, MDH spl CD4 and MD L g/p RRE. In both vectors, the gag/pol genes are framed by splice donor and splice acceptor sites and thus p24 is expressed when Rev is present in the culture.

#### **DETAILED DESCRIPTION OF THE INVENTION**

The invention takes advantage of the splicing control mechanisms of HIV and other lentiviruses which regulate expression of the late viral genes, gag/pol and env, by means of a cis acting RNA element, RRE, and a trans acting regulatory protein, Rev. By the strategic placement of splice control elements, a switch in a gag/pol expression construct allows expression of a downstream selectable marker gene in the basal state and of the upstream gag/pol genes only on induction. As both genes are driven by the same constitutive promoter, operation of the switch allows for gag/pol induction to an expression level related to that of the selectable marker.

Three features operate the switch: 1) the gag/pol genes are contained within a splice donor site and one or more splice acceptor sites, wherein the sequences of the acceptor sites do not match the optimal consensus splice acceptor sequence (Lewin, "Genes", John Wiley & Sons, NY) upstream of the marker gene; 2) the gag/pol genes contain sequences which antagonize the expression of gag/pol (Schneider et al., J. Virol. 71:4892-4903, 1997; Schwartz et al., J. Virol. 66:7176-7182, 1992); and 3) the gag/pol genes are linked in cis to the RRE element as well as being separated from the Rev coding sequence.

A promoter which controls the expression of both gag/pol and the marker gene is situated operably thereto, generally upstream from the gag/pol sequences.

The RRE/Rev regulatory system is found in lentiviruses and thus, that of HIV-1 or any other lentivirus can be used. Also, any other trans complementing regulatory system 5 which results in selective splicing which would control the expression of gag/pol as described herein can be used in the practice of the instant invention.

The first two features combine to suppress gag/pol expression in the basal state. The third feature allows for Rev-dependent stimulation, i.e., induction, of the export of unspliced RNA and consequent expression of the gag/pol genes.

10                 Regarding the splice sites, a combination of an efficient splice donor site, such as that of the 5' major splice donor of HIV, and one or more splice acceptor sites, wherein the splice acceptor sites do not match exactly the optimal consensus, are used. Therefore, the splice acceptors of interest are those an artisan would recognize as not being that efficient, strong or good. Nevertheless, the splice acceptor sites are operable, albeit at a suboptimal 15 rate of efficiency. The suboptimal splice sites appear to allow for more efficient expression from unspliced transcripts by the Rev-RRE system. An example of such a suboptimal splice acceptor site is that of the third exon of the HIV-1 tat and rev genes.

Non-lentivirus splice donor and splice acceptor sites also can be used in the 20 practice of the instant invention so long as the splicing, and hence expression, of the gag/pol genes is controlled by the presence of a trans acting factor, such as Rev.

The intrinsic instability of the lentiviral gag/pol coding sequences, and particularly the sequences contained in the intron, counteracts expression in the basal state from unspliced transcripts that may accumulate due to the suboptimal nature of the splice sites. Any sequence which is known to be associated with the instability of transcripts can be used in the

practice of the instant invention. Instability sequences, however, such as those identified described in Schneider et al. and Schwartz et al., *supra*, in the gag/pol sequence, may not be strictly required for the operation of the switch.

Any of a number of possible selectable markers can be used. Markers which are 5 readily detectable are desirable. For example, the marker may be a cell surface molecule, which is antigenic, such as a CD molecule or lymphocyte antigen, or a light-emitting molecule, such as green fluorescent protein. An artisan is free to select a selectable marker of interest from those known in the art.

The methods for cloning the various elements of the instant invention into a vector of 10 interest are known in the art.

As a means of introducing yet another level of regulation, expression of the trans acting splice regulatory elements, in the case of HIV-1, Rev, can be inducible as well. In the presence of a separate inducible Rev expression construct, the expression of the gag/pol genes becomes inducible. For example, expression of Rev can be inducible using the 15 tetracycline dependent regulatory system of Ory et al. (Proc. Natl. Acad. Sci. 93:11400-11406, 1996) wherein Rev is subcloned adjacent to a tet operator. In the presence of tet, Rev is not expressed. However, when tet is withdrawn from the medium, Rev expression occurs.

Other known regulatory elements can be used as known in the art. Thus, a 20 suitable and known promoter can be placed operably in the construct to regulate expression of the gag/pol and marker genes. Other regulatory elements, such as a polyadenylation site can be used as desired.

Moreover, various modifications can be made to any one element included in the vectors of interest to remove undesirable activities or to enhance desired activities. The

artisan can rely on the known activities of the elements contemplated and can practice known techniques to effect the desired changes, for example, deletion of sequences by selective subcloning, inactivation of a gene by site directed mutagenesis and so on.

An advantage of the instant invention is selection of optimal packaging clones 5 for vectors, such as lentiviral-derived, and particularly, HIV-derived, vectors. Using a surface marker for the linked selection, a population of stable, high-level expressors can be sorted on transfection of the constructs, and subsequently as often as needed to maintain performance. In previously described linked-selection systems, expression of the marker gene is coupled to the expression of the desired gene and cannot be operated in the reverse direction.

10 The instant method also can be used to select packaging clones for lentiviral vectors other than HIV-1, either using the HIV-1 Rev-RRE system, or homologous elements of other lentivirus, so long as the homologous regulatory elements functionally operate equivalently to yield selectable splicing of the gag/pol sequence in the presence of an inducer molecule located in trans to the coding sequences of interest.

15 The invention now will be exemplified in the following non-limiting examples.

## EXAMPLES

A packaging vector, pMDH L g/p RRE Sp1 CD4 (Figure 1) was constructed to include the following: immediate/early enhancer/promoter of the human cytomegalovirus 20 (CMV); HIV major 5' splice donor; HIV gag/pol coding regions with optimized translation initiation sequence fitting the Kozak consensus sequence (Dull et al. J. Virol. 72:8463-8471, 1998); HIV RRE element; HIV splice acceptor sites from the 3<sup>rd</sup> exon of tat and rev; human CD4 coding region; and rat insulin poly-adenylation sequence.

The lentiviral packaging vector pMDH L g/p RRE Spl CD4 allows for selection of high level expression of the surface marker CD4 with very low expression of the HIV-1 gag/pol genes. Due to the linkage of the CD4 marker to the gag/pol genes, high expression of CD4 correlates with high inducible expression of gag/pol. In the absence of 5 HIV Rev, splicing of the gag/pol sequences between the HIV splice donor and acceptors yield efficient expression of CD4 without appreciable expression of gag and pol (Figure 2A). In the presence of Rev, the RRE-mediated export of unspliced gag/pol message allows expression of the gag pol proteins (Figure 2B).

The pMDH L g/p RRE Spl CD4 plasmid was transfected into 293T (Dull et 10 al., *supra*) with or without a Rev expression plasmid (Dull et al., *supra*) and with a combination of other plasmids required to generate lentiviral vector delivery of a selectable marker, green fluorescent protein (GFP).

About  $4 \times 10^6$  293T cells were plated per 10 cm dish the night prior to transfection. CaPO<sub>4</sub> co-transfection of the following plasmids was performed: pMDH L g/p 15 RRE Spl CD4, 7 $\mu$ g (HIV-derived gag/pol expression plasmid); pRSV Rev, 2.5 $\mu$ g; pCMV tat, 1 $\mu$ g; pMD VSVG env, 3.5 $\mu$ g; and pRRLhPGKGFP SIN-18, 10 $\mu$ g (a self-inactivating HIV-derived transfer vector carrying a green fluorescent protein coding sequence linked to a PGK promoter). Identical transfections also were performed without the pRSV Rev plasmid, and with the parental packaging vector pMD L g/p RRE in place of pMDH L g/p RRE Spl CD4. 20 Twenty hours after transfection, fresh medium was added and 24 hours later, conditioned medium was harvested for measuring the content of the HIV gag product, p24, by immunocapture (Dupont) and for assaying transduction. The transfected cells were harvested, incubated with phycoerythrin-labelled anti-CD4 antibodies and analyzed by FACS for phycoerythrin and GFP fluorescence.

The transfectants were analyzed for expression of both CD4 and GFP, with and without HIV Rev. In both cases the vast majority of cells were doubly positive for CD4 and GFP. As expected, the average expression level of CD4 was higher in cells not expressing Rev. Expression of basal levels CD4 in the presence of Rev is due to the fact that Rev does not prevent completely the splicing of RRE-containing transcripts.

Similar transfections also were performed with the parental packaging vector pMD L g/p RRE in place of pMDH L g/p RRE Sp1 CD4. The vector pMD L g/p RRE expresses gag/pol of HIV in a Rev-dependent manner downstream of a constitutively spliced intron derived from the  $\beta$ -globin gene. Cells co-transfected with the pMD L g/p RRE packaging vector and Rev expressed gag/pol whereas in the absence of Rev, no gag/pol was detected.

Expression of the gag/pol genes in the transfected 293T cells was analyzed by measuring the content of the gag gene product, p24, in the conditioned medium by immunocapture (DuPont). Figure 3 shows the p24 concentration in the conditioned media of cells transfected with both packaging vectors in the presence and absence of HIV Rev. The Rev dependence of gag/pol expression for both plasmid is evident. The plasmid which contains the CD4 coding sequence expresses a very high level of p24 protein in the presence of Rev, similar to that obtained with the control plasmid.

Production of functional vector was analyzed by using the 293T conditioned medium to transduce the GFP gene into HeLa cells. HeLa cells were exposed to 10  $\mu$ l of medium conditioned by cells transfected with the pMDH L g/p RRE Sp1 CD4 packaging vector or the pMD L g/p RRE packaging vector in the presence (a) and absence (b) of HIV Rev. Transduction experiments were carried out by plating  $5 \times 10^4$  cells/well in 6-well plates the night prior to infection. The next day, frozen 293T conditioned medium was thawed and diluted 1:10, 1:100, 1:1000, and 1 ml of each dilution was used to infect the cells. Twenty

hours after infection, fresh medium was added and 24 hours later, cells were analyzed by FACS for GFP expression.

The Rev-dependence of the transduction was evident for both plasmids. Only when Rev is expressed in vector producer cells do the target HeLa cells express GFP. Moreover, 5 the infectivity (transducing units/ng p24) of vector produced by either plasmid is similar, indicating that the CD4-linked plasmid operates as efficiently as the control plasmid.

**We claim:**

1. A nucleic acid construct comprising in operable linkage in the 5' to 3' direction;

(1) a promoter;

(2) a splice donor site;

(3) a gag/pol coding sequence;

5 (4) a Rev responsive element or functional equivalent thereof;

(5) a splice acceptor site; and

(6) a selectable marker coding sequence.

2. A composition comprising:

(a) a first expression cassette comprising in operable linkage in the 5' to 3' direction:

(1) a promoter;

(2) a splice donor site;

5 (3) a gag/pol coding sequence;

(4) a Rev responsive element or functional equivalent thereof;

(5) a splice acceptor site; and

(6) a selectable marker coding sequence; and

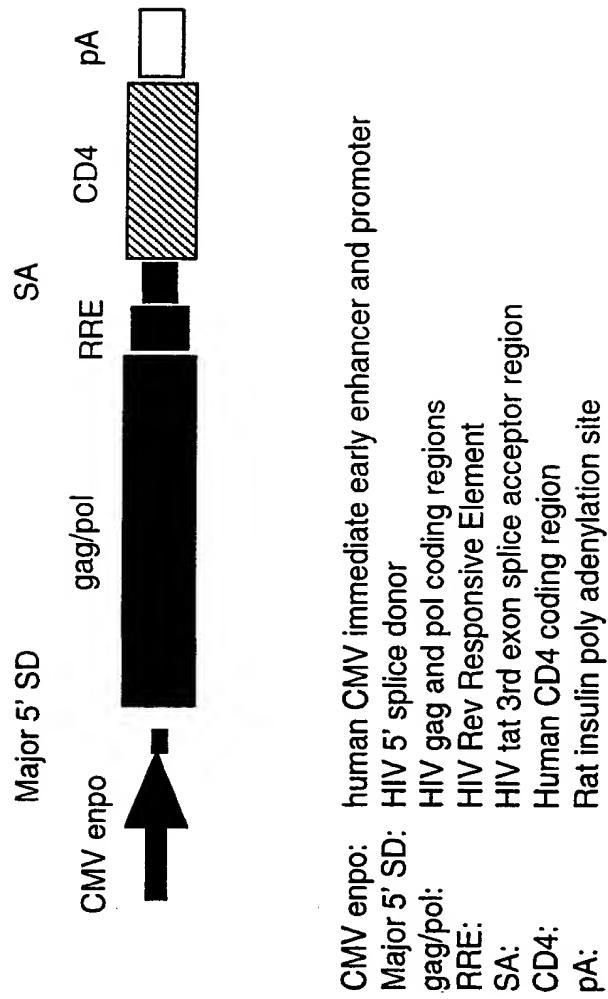
(b) a second expression cassette comprising in operable linkage in the 5' to 3'

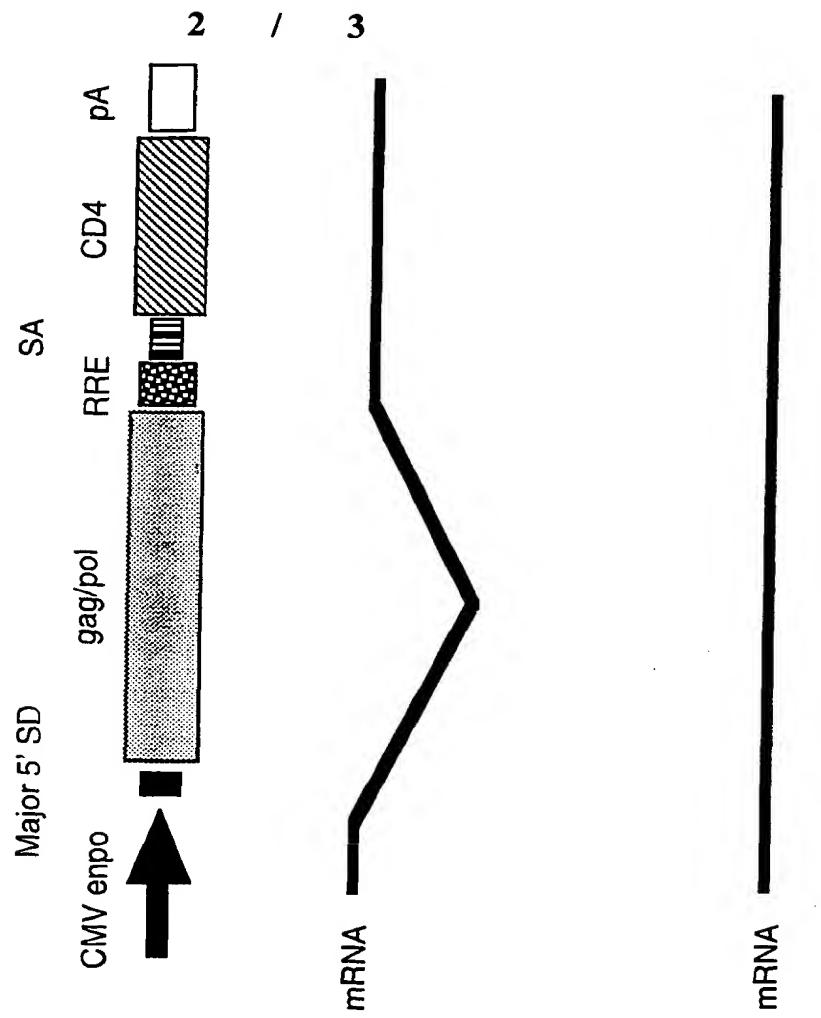
10 direction:

(1) a promoter; and

(2) a nucleic acid encoding a factor which binds to element (4) of said first expression cassette, which on such binding regulates splicing at said sites (2) and (5) of said first expression cassette when an mRNA is transcribed from said first expression cassette.

Figure 1 pMDH L g/p RRE SpI CD4

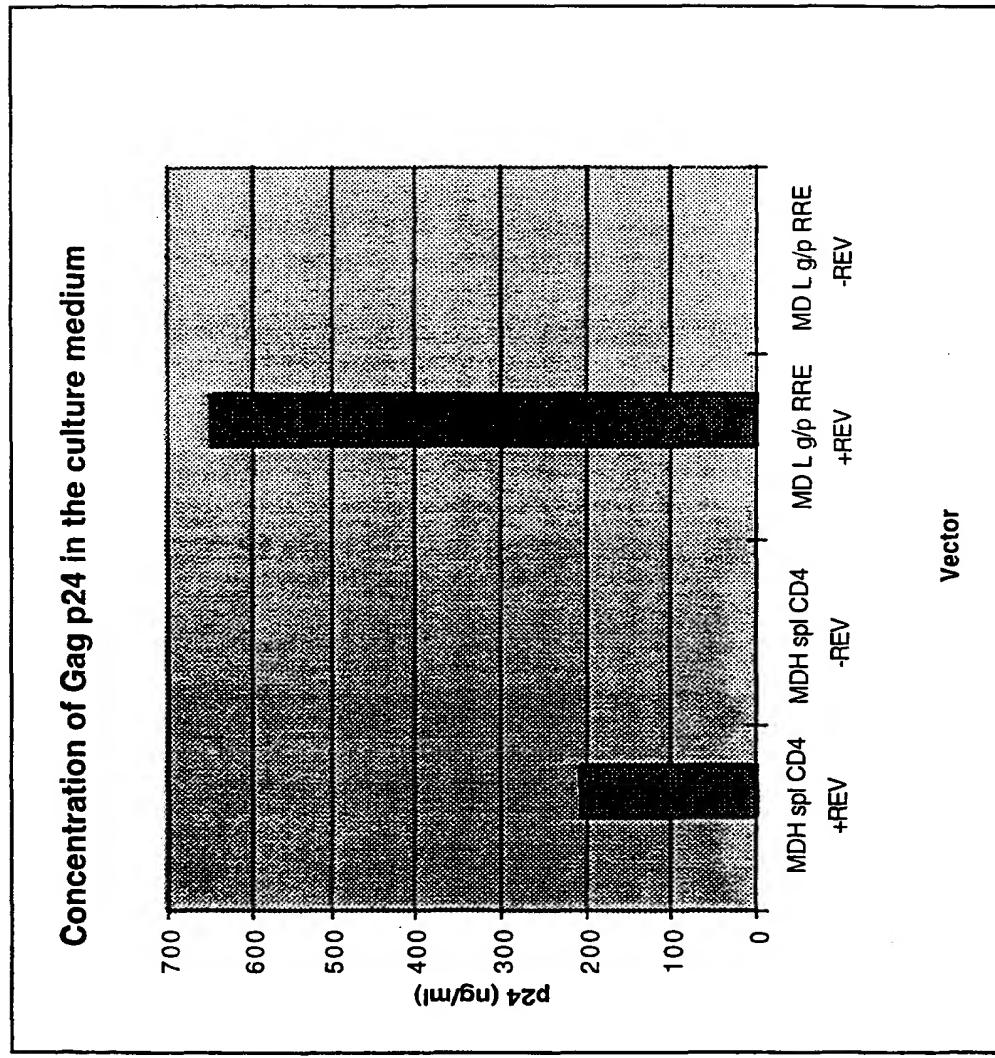


**Figure 2**

A. Absence of Rev permits splicing to occur between the splice donor and acceptors, thus eliminating gag/pol sequences and expression of their gene products. CD4 is efficiently expressed from this messenger RNA.

B. Presence of Rev suppresses splicing resulting in inclusion of gag/pol sequences and expression of their gene products.

Figure 3



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/24018

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07H 21/00, 21/04; C12N 15/00, 15/09, 15/63, 15/70, 15/74  
US CL : 435/320.1; 536/23.1, 23.7, 23.72

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1; 536/23.1, 23.7, 23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, DIALOG, EMBASÉ, MEDLINE, BIOSIS, SCISEARCH  
search terms:nucleic acids, construct, promoter, donor site, gag, pol, HIV, marker, expression cassette, vector, REV

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,739,118 A (CARRANO et al) 14 April 1998, columns 33-34.	1 and 2
X	US 5,716,613 A (GUBER et al.) 10 February 1998, columns 11-12.	1 and 2
X	US 5,716,826 A (GRUBER et al.) 10 February 1998, columns 11-12.	1 and 2
Y	US 5,747,307 A (LEVER et al.) 05 May 1998, figs. 4-5, columns 7-12.	1 and 2
Y	US 5,693,508 A (CHANG) 02 December 1997, columns 45-48.	1 and 2

 Further documents are listed in the continuation of Box C.

See patent family annex.

Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 FEBRUARY 2000

Date of mailing of the international search report

15 FEB 2000

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

BRETT L. NELSON

Telephone No. (703) 308-0196

PATENT COOPERATION TREATY

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: DEAN H. NAKAMURA  
ROYLANCE, ABRAMS, BERDO & GOODMAN, LLP  
1300 19TH Street, N.W., Suite 600  
WASHINGTON DC 20036

PCT

ROYLANCE, ABRAMS,  
BERDO & GOODMAN, LLP

18 2000

NOTIFICATION OF TRANSMITTAL OF  
INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing  
(day/month/year)

12 JAN 2001

Applicant's or agent's file reference  
P134322

IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority Date (day/month/year)
PCT/US99/24018	12 NOVEMBER 1999	13 NOVEMBER 1998

Applicant

CELL GENESYS, INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231  
Facsimile No. (703) 305-3230

Authorized officer  
BRETT L NELSON  
TERRY J. DEY  
PARALEGAL SPECIALIST  
TECHNOLOGY CENTER 1800  
Telephone No. (703) 308-0196

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

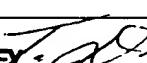
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference F134322	FOR FURTHER ACTION      See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US99/24018	International filing date (day/month/year) 12 NOVEMBER 1999	Priority date (day/month/year) 13 NOVEMBER 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant CELL GENESYS, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets.
 

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 0 sheets.
3. This report contains indications relating to the following items:
  - I  Basis of the report
  - II  Priority
  - III  Non-establishment of report with regard to novelty, inventive step or industrial applicability
  - IV  Lack of unity of invention
  - V  Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
  - VI  Certain documents cited
  - VII  Certain defects in the international application
  - VIII  Certain observations on the international application

Date of submission of the demand 31 MAY 2000	Date of completion of this report 04 JANUARY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BRETT L NELSON Telephone No. (703) 308-0196
 <b>TERRY J. DEY</b> <b>PARALEGAL SPECIALIST</b> <b>TECHNOLOGY CENTER 1600</b>	

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/24018

## I. Basis of the report

## 1. With regard to the elements of the international application:\*

 the international application as originally filed the description:pages 1-8 \_\_\_\_\_, as originally filed  
pages NONE \_\_\_\_\_, filed with the demand  
pages NONE \_\_\_\_\_, filed with the letter of \_\_\_\_\_ the claims:pages 9-10 \_\_\_\_\_, as originally filed  
pages NONE \_\_\_\_\_, as amended (together with any statement) under Article 19  
pages NONE \_\_\_\_\_, filed with the demand  
pages NONE \_\_\_\_\_, filed with the letter of \_\_\_\_\_ the drawings:pages 1-3 \_\_\_\_\_, as originally filed  
pages NONE \_\_\_\_\_, filed with the demand  
pages NONE \_\_\_\_\_, filed with the letter of \_\_\_\_\_ the sequence listing part of the description:pages NONE \_\_\_\_\_, as originally filed  
pages NONE \_\_\_\_\_, filed with the demand  
pages NONE \_\_\_\_\_, filed with the letter of \_\_\_\_\_

## 2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item. These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

 the language of a translation furnished for the purposes of international search (under Rule 23.1(b)). the language of publication of the international application (under Rule 48.3(b)). the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

## 3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

 contained in the international application in printed form. filed together with the international application in computer readable form. furnished subsequently to this Authority in written form. furnished subsequently to this Authority in computer readable form. The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished. The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.4.  The amendments have resulted in the cancellation of: the description, pages none the claims, Nos. none the drawings, sheets/fig none5.  This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\*Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. statement**

Novelty (N)	Claims <u>none</u>	YES
	Claims <u>1-2</u>	NO
Inventive Step (IS)	Claims <u>none</u>	YES
	Claims <u>1-2</u>	NO
Industrial Applicability (IA)	Claims <u>1-2</u>	YES
	Claims <u>none</u>	NO

**2. citations and explanations (Rule 70.7)**

Claims 1 and 2 lack novelty under PCT Article 33(2) as being anticipated by Guber et al. (U.S. Pat. No. 5,716,613). The claims are drawn to a construct comprising a promoter, a splice donor site, a gag/pol coding sequence, a REV responsive element, a splice acceptor site, and a selectable marker coding sequence and a composition comprising the first construct and a second construct comprising a promoter and a nucleic acid encoding a factor which binds to the REV responsive element of the first construct. Guber et al. disclose a composition for expressing gag and pol comprising a CMV promoter, a splice donor site, a gag/pol coding sequence, a REV responsive element, a splice acceptor site, and a selectable marker coding sequence such as Neo and a construct comprising a promoter and a nucleic acid encoding a factor which binds to the REV responsive element of the first construct (cols. 11 and 12). The composition of Guber et al. is the same as the claimed composition. Therefor ore, Guber et al. anticipate the claimed invention.

Claims 1 and 2 lack novelty under PCT Article 33(2) as being anticipated by Gruber et al. (U.S. Pat. No. 5,716,826). The claims are drawn to a construct comprising a promoter, a splice donor site, a gag/pol coding sequence, a REV responsive element, a splice acceptor site, and a selectable marker coding sequence and a composition comprising the first construct and a second construct comprising a promoter and a nucleic acid encoding a factor which binds to the REV responsive element of the first construct. Gruber et al. disclose a composition for expressing gag and pol comprising a CMV promoter, a splice donor site, a gag/pol coding sequence, a REV responsive element, a splice acceptor site, and a selectable marker coding sequence such as Neo and a construct comprising a promoter and a nucleic acid encoding a factor which binds to the REV responsive element of the first construct (cols. 11 and 12). The composition of Gruber et al. is the same as the claimed composition. Therefore, Gruber et al. anticipate the claimed invention.

(Continued on Supplemental Sheet.)

**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

**CLASSIFICATION:**

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): C07H 21/00, 21/04; C12N 15/00, 15/09, 15/63, 15/70, 15/74 and US CL.: 435/320.1; 536/23.1, 23.7, 23.72

**V. 2. REASoNED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):**

Claims 1-2 meet the criteria set out in PCT Article 33(4), because the claimed invention has industrial applicability in the field of Biotechnology.

**----- NEW CITATIONS -----**

NONE

**PATENT COOPERATION TREATY**

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

Doc'd

Rec'd

KD File 397169  
JUL 27 2000

PCT

ROYLANCE, AERAMS  
BERDO & GOODMAN, L.L.P.  
BY

NOTIFICATION OF RECEIPT  
OF DEMAND BY COMPETENT INTERNATIONAL  
PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence  
and Administrative Instructions, Section 601(a))

Date of mailing  
(day/month/year)

**21 JUL 2000**

Applicant's or agent's file reference <b>F134322</b>		IMPORTANT NOTIFICATION	
International application No. <b>PCT/US99/24018</b>	International filing date (day/month/year) <b>12 NOV 99</b>	Priority date (day/month/year) <b>13 NOV 98</b>	
Applicant <b>CELL GENESYS, INC.</b>			

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

**31 May 00**

2. That date of receipt is:

the actual date of receipt of the demand by this Authority (Rule 61.1(b)).  
 the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).  
 the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3.  **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

*(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/ Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn: RO/US Facsimile No. 703-305-3230	Authorized officer <b>Vanessa Clark</b>  Telephone No. 703-305-6485
---	--